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Genetic Activity of Sex Chromosomes in Somatic Cells of Mammals

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Genetic activity of sex chromosomes in somatic cells of mammals*

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[Plate 4]

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Mammals are thought to have a type of dosage compensation not so far known in any other animal group: however many X chromosomes are present, only one remains genetically active in somatic cells. Considerable evidence for this idea exists, in spite of criticism; the greatest difficulty is presented by the abnormalities in human individuals with X chromosome aberrations. Possible explanations for these abnormalities include: wrong X chromosome dosage in early development before X inactivation, reversal of inactivation, partial inactivation of both X chromosomes, activity of the X while in the condensed inactive state, and the presence of a homologous non-inactivated region of the human X and Y. In female germ cells X inactivation apparently does not occur, but the situation in male germ cells is less clear. The Y chromosome is probably also inactive in somatic cells of adults, but again its function in germ cells is not yet clear. Some species have a presumed doubly inactive X chromosome region, as well as the singly active one. The origins and functions of this region are unknown; it may have a role in female germ cells.

Introduction

Many unusual sex chromosome systems are known in mammals (Fredga, this volume, p. 15). Another phenomenon associated with the sex chromosomes, in which mammals are unusual in relation to other animal groups but apparently fairly consistent among themselves, is in their system of regulating the activity of genes on the sex chromosomes. In the typical mammalian XX–XY sex chromosome system the X is usually larger than the Y, and carries many genes whose functions are apparently quite unconnected with sex. The human X, for instance, carries genes concerned with haemophilia, muscular dystrophy, colour blindness, skin diseases, and the Xg blood group, among many others (McKusick 1962), and the mouse X carries genes for coat colour, tail kinks, a type of anaemia, and so on. Since the Y chromosome does not carry any corresponding genes the question arises how the potentially different amounts of X-linked gene products in the two sexes are integrated with a constant amount from the autosomal genes. This problem exists in many animal groups which have an X–Y or Z–W sex determining system and it appears that various methods of dosage compensation for dealing with it have been evolved

In memory of Dr Margaret M. Dickie who died 4 July 1969.

in different groups (Stern 1960; Cock 1964), and that the mammals have a system, which as far as is known, is unique to them. The system is thought to be that, however many X chromosomes are present in a somatic cell, only one remains genetically active and forms messenger RNA. The others, normally one in a female and none in a male, become inactive and form the sex chromatin body found lying against the nuclear membrane (Barr & Bertram 1949). Appreciation of this point is important for a better understanding of sex determination in mammals and this paper discusses the evidence for X inactivation and its relation to sex chromosome abnormalities.

The hypothesis was first put forward as an explanation of the variegated phenotype of female mice heterozygous for X-linked colour genes (Lyon 1961). The variegation is similar to that seen in tortoiseshell cats and the tortoiseshell hamster (Robinson 1966) where again the colour difference is due to an X-linked gene. The male, with his single X, can be either light coloured or normal coloured, whereas in the female there are three possibilities: (i) both X chromosomes with genes for normal colour, or (ii) both for light colour, when the effect is exactly like that in males, or (iii) one gene of each kind on the two X chromosomes, when the female has patches of the two colours.

The explanation put forward to account for this was that one of the two X chromosomes of the female becomes inactive early in embryonic development. Once the inactivation has occurred the same X remains inactive throughout the further development of any particular cell line, so that in the adult there are clones of cells with the same X active, each descended from one cell at the time of inactivation. If the two X chromosomes have genes for different coat colours then the cells with the light colour gene active will give a light coloured patch, and those with the dark colour gene active a dark one.

Evidence concerning X inactivation

Phenotypes of heterozygotes

Considerable evidence in favour of X inactivation has now been accumulated (Lyon 1966*a*, 1968), but the hypothesis has also been criticized, particularly by Grüneberg (1966, 1967*a*, *b*) who claimed that the detailed phenotypes of the variegated heterozygotes were not in accord with expectation. These criticisms have been answered in a recent paper (Lyon 1968) in which it was pointed out that in order to formulate expectations concerning the sizes, shapes and distribution of patches it is necessary not to compare effects in different cell lineages, but rather those produced in the same cell type by different mechanisms. In particular, patterns produced by genes acting through pigment cells or through hair follicle cells could be compared in variegated mice resulting from X inactivation or from experimental egg fusion (Mintz 1967; Mystkowska & Tarkowski 1968; Gardner 1968). The argument developed there can now be carried slightly further.

Genes acting through the hair follicle cells (e.g. tabby, striated, and agouti in the mouse), produce, both after X inactivation and after egg fusion, a pattern of marked transverse stripes. This calls to mind the somites, and if the genes were acting through the mesodermal rather than the ectodermal cells of the hair follicle, one would indeed expect any variegated pattern produced by them to be related to the somites. For tabby, there is reason to suppose that it does act through mesodermal cells, as previously suggested (Lyon 1966*b*), because it affects number and shape of tooth cusps, and Kollar & Baird (1969) have recently shown experimentally

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that cusp shape in the mouse is determined by the mesodermal component of the tooth germ. Pigment cells, on the other hand, are derived from the neural crest, and as this originates as groups of cells lying between the somites, one would expect pigment cell pattern also to bear some relation to the somites. However, in pigment cell variegation the transverse element, though present, is much less marked, and there are many small patches, much smaller than a somite. A possible explanation for this would be that any given group of inter-somite neural crest cells is derived not from a single foundation cell at the time of X inactivation or egg fusion, but from two or more such cells. If two, then some groups would be derived from two dark or two light foundation cells and would result in broad transverse dark or light stripes. Others would be derived from one dark and one light cell. The dark and light descendant cells would mingle as they migrated out, and would then each settle to form a small patch. This would account for the many small patches. A similar but more complicated argument could be developed for the case when more than two foundation cells contribute to a single group of neural crest cells.

It is possible now to produce simulated patterns, for genes acting through somite or neural crest cells, based on expectations for various numbers of foundation cells contributing to a somite or inter-somite group, and a random choice of foundation cells of two types. Figure 1 shows some such simulations. To obtain these patterns, the number of somites from the head to the base of the tail of a mouse was taken to be 35, and it was assumed that the right and left sides were derived independently from the foundation cells. Twenty-five white and twenty-five black beads were put into a bag, which was well shaken. Then according to the particular simulation one, two or three beads were withdrawn with the left and right hands separately, the results were noted, and the beads were returned to the bag, which was again shaken. The procedure was repeated for 35 withdrawals, to give one simulation.

The postulate of one foundation cell per somite gave a pattern fairly similar to that seen in heterozygotes for the tabby, brindled or blotchy genes, but quite different from that in heterozygotes for Cattanach's (1961*a*) translocation, where the variegation is due to genes (albino or pink-eye) acting through pigment cells. A pattern resembling that of Cattanach's heterozygotes was, however, produced by a postulate of two cells per somite. The features which the patterns in the mice and the simulations had in common were (i) a detectable but not marked transverse element, (ii) a tendency to discontinuity at the mid-line, and (iii) a wide variation in size of patch, too great to be accounted for by chance variation in numbers of contiguous unit patches of the same colour type. These same features also appear in the simulations using a postulate of three foundation cells per somite. In fact there are no obvious distinctive differences between the simulations using two and three cells per somite, and it is clear that this method cannot be used at present to determine exactly how many foundation cells contribute to one inter-somite group. Moreover, it is probably not correct to assume that migrating melanoblasts are restricted in distribution to the area of one somite; mingling of cells from adjacent groups may well occur, and would tend to blur the pattern. Indeed, if much mingling is assumed similar patterns might well be obtained with a postulated number of initial groups much less than the number of somites. The simulation suffices to show, however, that a pattern resembling that seen after X-inactivation or egg fusion can be obtained by assuming: (i) a random arrangement of cells, rather than the regular alternation suggested by Mintz (1967), and (ii) a number of initial cell groups much higher than that suggested by Mintz.

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The last group of simulations, using a postulate of one foundation cell per two somites, resulted in a pattern fairly similar to that in mice both heterozygous for Cattanach's translocation and homozygous for piebald spotting (*ss*). As with the tortoiseshell cat (Norby, Thuline & Priest 1962) and tortoiseshell hamster, the variegated patches are larger in animals with the autosomal spotting gene (figure 2, plate 4). The simulation shows that this could be due to the spotting gene causing a reduction in number of primordial neural crest cells. It could also be due, however, to reduced mingling or migration of melanoblasts.

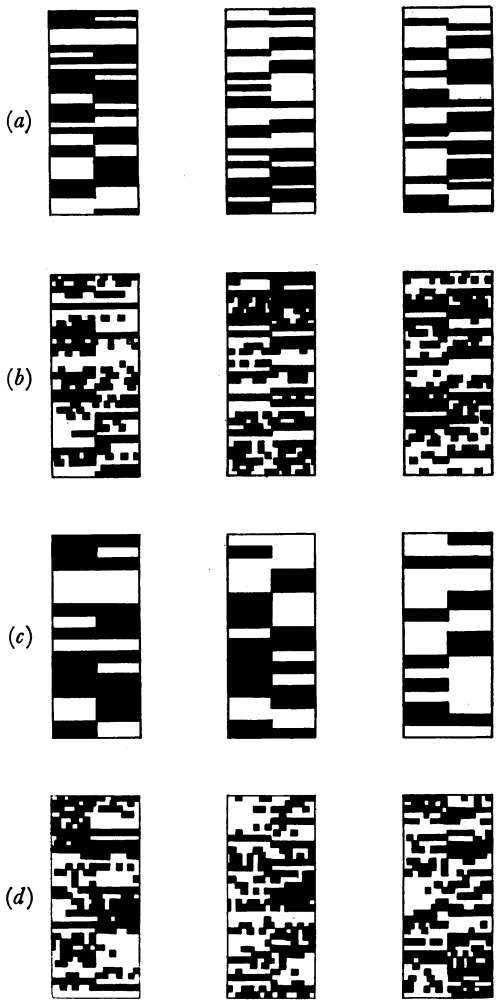

FIGURE 1. Patterns resulting from some simulated random arrangements of foundation cells for genes acting through the somites or inter-somite groups. (*a*) One foundation cell per somite, (*b*) two foundation cells per somite, (*c*) one per two somites, and (*d*) three per somite.

Alternate action in double heterozygotes

Other evidence about the hypothesis concerns the appearance of females heterozygous for two non-allelic X-linked genes. If the two genes act through the same cell type they should show alternate action, with patches showing only one or other gene effect if the genes are on different X chromosomes, and both or neither if they are on the same X. This has been tested in the mouse with various pairs of genes which affect coat colour or texture. One pair was tabby (*Ta*) and striated (*Str*) which affect hair texture. Striated heterozygotes have patches of abnormally short hair, whereas tabbies have patches with very few of the zigzags which give the normal

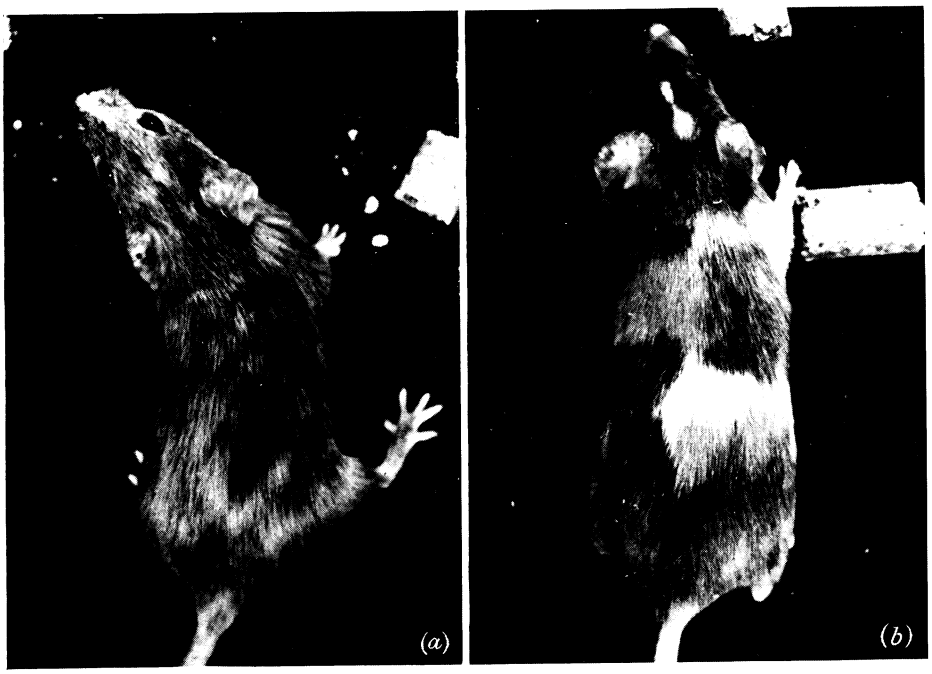

FIGURE 2. Female mice heterozygous for Cattanach's translocation and variegated for pink-eye (*p*). (*b*) is also homozygous for piebald spotting (*s*). Note larger size of variegated patches in (*b*).

(*Facing p.* 44)

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coat its fluffy texture. When samples of hair were taken from heterozygotes having striated and tabby on opposite chromosomes the hair in the short patches, where *Str* was acting, had a nearly normal proportion of zigzags, whereas that from the normal length patches had very few zigzags. Conversely, in animals with *Str* and *Ta* on the same X the short patches had very few zigzag hairs, and the normal length ones had a near normal proportion (figure 3). Neither type of heterozygote had the naked tails and bald patches behind the ears seen by Gruneberg (1967*b*). Thus, the two genes were acting alternately.

In addition to *Ta* and *Str*, Lyon (1963) presented evidence about the gene pair *Ta* and Mo^{dp}

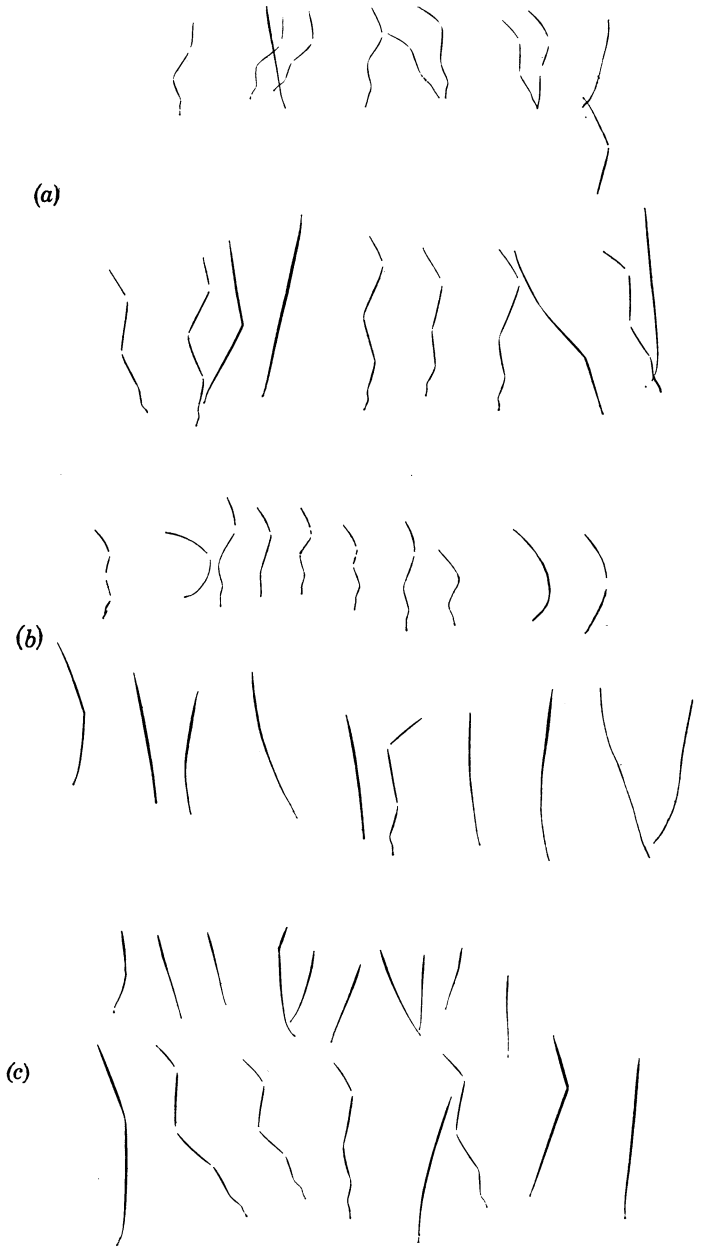

FIGURE 3. Samples of hairs from striated and tabby heterozygotes (*a*). *Str*/+, with equal proportions of zigzags in short and normal length samples. (*b*) *Str*+/+ *Ta*, with few zigzags in normal length sample. (*c*) *Str Ta*/++, with few zigzags in short sample.

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(dappled) which did not show alternate action, and about Mo^{dp} and *p* variegation in Cattanach's translocation, which did. Data can now be presented on the pairs *Ta–*Mo^{br} and *Ta–Blo*. Brindled (Mo^{br}) and blotchy (*Blo*) both cause light patches in the coat, but no lightening of the skin pigment in ears and tail, and their hair-lightening effect is thought to be due to some effect on hair structure. Like *Str* they do not alter the proportion of zigzag hairs in the coat. Table 1 shows that in *Ta*+/+Mo^{br} and *Ta*+/+*Blo* the effect of *Ta* in reducing frequency of zigzags was seen only in the dark patches where Mo^{br} and *Blo* were not acting, whereas in *Ta* Mo^{br}/++ and *TaBlo*/++ the effect was reversed. As Mo^{br} and *Blo* act alternately with *Ta* one would expect them not to act alternately with Cattanach's translocation. This is indeed so. Cattanach (1963) found that Mo^{br} did not show alternate action with the *c* variegation of his translocation and *Blo* similarly does not with the *p* variegation (Lyon, unpublished). The one exceptional pair are Mo^{dp} and the *c* variegation in Cattanach's translocation. As Mo^{dp} acts alternately with *p* in heterozygotes for this translocation one would expect it also to do so with *c*. However, Cattanach (1966) found that it did not, and the reason for this discrepancy is not known, but may be connected with incomplete inactivation of the translocated segment.

TABLE 1. HAIR COUNTS FROM HETEROZYGOTES FOR *Ta*, Mo^{br} AND *Blo*

genotype	number of mice	sample region	number of hairs: zigzag	awl	auchene	guard hair	% zigzag hairs
Mo^{br}/+	2	light	108	66	14	4	56.3
		dark	128	46	8	3	69.2
Ta+/+Mo^{br}	9	light	475	265	76	12	57.4
		dark	79	826	13	9	8.5
TaMo^{br}/++	2	light	31	191	5	4	13.4
		dark	136	26	12	1	77.7
Blo/+	2	light	162	31	20	—	76.1
		dark	157	38	16	1	74.1
Ta+/+*Blo*	9	light	516	237	47	10	63.7
		dark	141	674	16	8	16.8
TaBlo/++	9	light	180	741	13	7	19.1
		dark	509	280	40	7	60.9

The importance of the alternate action test is that it shows that the different genes are not acting independently in showing single allele action. Rather, this is a chromosomal effect since points some distance apart on the chromosome are correlated in activity. Secondly, it is difficult to think of alternative explanations for this type of result. Grüneberg (1967*b*) claims that the effects are not in fact due to alternate action, but to some kind of physiological interaction between the genes concerned. However, he offers no explanation as to why this should depend on the *cis-trans* relations of the genes on the chromosomes.

Non-random inactivation

Another line of evidence in the mouse, again concerned with chromosomal behaviour, involves the effect on gene expression of a chromosome aberration causing non-random inactivation. The patchy effect usually seen in heterozygotes for X-linked genes depends on the random inactivation of one or other X. If the same X is active in all cells one would instead expect to see the full action of any genes on the active X and no effect of those on the inactive X. In heterozygotes for Searle's translocation (Lyon, Searle, Ford & Ohno 1964), the X involved in the translocation is thought to be active in all cells and the normal X inactive. As expected,

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genes on the active X show the full effect, as in a male, and those on the inactive X are not expressed. Again one can show this simultaneously with genes a considerable distance apart on the chromosome (Lyon 1966*b*; and see below) and again it is difficult to think of other explanations. This point is also of interest in relation to human X chromosome aberrations. Human females can be found who have one normal and one structurally abnormal X. Commonly they have non-random X inactivation and any X-linked genes that they carry are expressed as in a male.

X inactivation and sex chromosome aberrations

Although there is plenty of evidence in favour of the hypothesis, there are still difficulties, and various suggestions have been made that, in one way or another, the inactivation is incomplete. Some of the greatest difficulty is presented by the abnormalities in human individuals with sex chromosome aberrations. Individuals with abnormal numbers of X chromosomes have one fewer sex chromatin bodies in their cells than they have X chromosomes (Barr 1966). If each sex chromatin body represents an inactive X, this means that however many X chromosomes are present, only one is genetically active, and one would therefore expect such individuals to be normal. In the mouse the XO type is indeed a normal fertile female, (Welshons & Russell 1959; Cattanach 1962; Morris 1968), but human XO types are sterile and have various abnormalities. Moreover, individuals with supernumerary X chromosomes, such as XXX, XXXX, XXY, XXXY, etc. are often mentally defective (Jacobs 1966) and their stature and fingerprint patterns (Penrose 1967) vary with the number of X chromosomes present. What possible explanations can be put forward?

X chromosome dosage in early development

One possibility is that wrong dosage of the X chromosome in early development, before the time of inactivation, leads to abnormality. It is known that the X chromosome is important in early development because Morris (1968) studied the development of mice lacking both X chromosomes, so-called OY mice, and found that they became abnormal and died after only one cleavage division. Hence at least one X is essential for development after the 2-cell stage. However, the sex chromatin body is not seen until the blastocyst or the primitive streak stage in man (Austin 1966). Therefore one must assume that until then all X chromosomes present are active and that any deviation from the normal XX or XY constitution may interfere with normal development. In line with this idea, many human XO types die and are aborted as early embryos (Carr 1967) and some mouse XO embryos also die. However, it seems unlikely that such things as stature and fingerprint patterns would be determined so early in development and one prefers to seek other explanations for variations in these.

Reversal of inactivation

Cattanach (1968) and Cattanach, Pollard & Perez (1969) have studied mice carrying his translocation and which are variegated for albino. He finds that in old animals brown hairs sometimes appear in the centre of white patches and he suggests that X inactivation can be reversed. He further finds that 'controlling elements' in the X chromosome affect the completeness of inactivation of the translocated X and he suggests that these elements may be affecting the frequency of reversal, and that reversal can occur in normal X chromosomes, as well as in the translocation.

If there were normally occasional reversal of inactivation, so that both X chromosomes were

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active in the same cell, then a normal female would have slightly more X activity than an XO. If the probability of one daughter cell being reversed at any cell division is r, and if the reversal is permanent, then after n successive cell divisions during embryogeny the proportion of tissue remaining with only single X activity would be $(1-\frac{1}{2}r)^n$. If r were $\frac{1}{1000}$ then after 50 and 100 successive cell divisions respectively, 2.4 and 4.7 % of the body would have two active chromosomes. If r were $\frac{1}{100}$, the corresponding figures would be 22.2 and 39.5 %. There is some doubt, however, whether reversal of inactivation does in fact occur. Comings (1966), Demars (1968) and Salzmann, Demars & Benke (1968) have looked for evidence of reversal in cultured human fibroblasts and have not yet found any. Comings thought that his method could have detected as little as 5 % activity of the second X, and Demars's method could detect single cells, while Salzmann *et al.* were able to suggest an upper limit of less than 10^{-6} per cell division for activation of the second X.

TABLE 2. PHENOTYPES OF MICE HETEROZYGOUS FOR SEARLE'S TRANSLOCATION AND CARRYING VARIOUS GENES ON THE ACTIVE AND INACTIVE X CHROMOSOMES

genes carried		
active	inactive	phenotype
+	*Blo*, *Bn*, *Gs*, *Mo*^{br}, *spf*, *Ta*, *Blo Gs*, *Bn Ta*, *Blo Ta*	+
Blo	*Ta*, *Bn Ta*	*Blo*
Gs	*Blo*, *Ta*	*Gs*
spf	+, *Blo*, *Ta*	*spf*
Ta	*Blo*	*Ta*
Blo Ta	+	*Blo Ta*
Ta	Cattanach's (with c^e c^e on autosomes)	*Ta* with flecks of wild-type colour on c^e c^e background.

Reversal, if it occurred, might also be detectable in mice heterozygous for Searle's translocation, which causes non-random inactivation, with the translocated X active in all cells. When such mice made homozygous for albino carry Cattanach's translocation on the inactive X, they have small patches of dark pigment on an albino background, indicating some activity of the wild-type allele of albino carried by the X with Cattanach's translocation (Cattanach 1966). The patches are smaller than those in simple heterozygotes for Cattanach's translocation (B. M. Cattanach, personal communication) which is as would be expected if they were formed by reversal of inactivation at any period of development, rather than at a restricted time in embryogeny, as with X inactivation or egg fusion. However, no such signs of reversal have yet been seen when X-linked genes, rather than Cattanach's translocation, are carried on the inactive X of females with Searle's translocation. Numerous different gene combinations have now been studied (table 2) and the double heterozygote with Cattanach's translocation is the only exception to the rule that the phenotype is like that of a homozygote or hemizygote for whatever genes are carried on the active X. The possibility that reversal is occurring undetected cannot yet be excluded, however, as none of the other genes studied is thought to act through pigment cells, and it is possible that very small patches of reversal in the other cell types concerned would go undetected. Further development of the simulations mentioned earlier might enable one to predict the frequency of occurrence of patches of reversal sufficiently large not to escape detection.

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Incomplete inactivation

Next there are various possible types of incomplete inactivation. Gruneberg (1967*b*) has suggested that, rather than one X being completely inactivated, both X chromosomes are partly inactive. The difficulty is that this would appear not to account for the cytologically visible differentiation of the X chromosomes, i.e. the condensation of the inactive X at interphase to form the sex chromatin body, its late replication, and heteropyknosis at mitotic prophase (reviewed by Lyon 1968). Nor would it account for the behaviour of single cell clones of cultured fibroblasts, which have been shown to exhibit activity of only one allele or other of the two possessed by heterozygotes for X-linked genes for glucose-6-phosphate dehydrogenase (G-6-PD) (Davidson, Nitowsky & Childs 1963; Demars 1968) hypoxanthine-guanine-phosphoribosyl-transferase (Migeon, Kaloustian, Nyhan, Young & Childs 1968; Rosenbloom, Kelley, Henderson & Seegmiller 1967; Salzmann *et al.* 1968), and X-linked Hurler's syndrome (Danes & Bearn 1967).

Russell (1963) suggested that in the mouse X the inactivation spread out from a certain point on the X and that there was a limited spread and therefore a gradient of activity along the X. Undoubtedly, the spread of inactivation is limited in autosomal segments involved in X–autosome translocations. There is as yet no positive evidence, however, that this phenomenon occurs in normal mouse X chromosomes (Lyon 1966*b*).

Hamerton (1968) suggests that the X chromosomes have some function as controlling elements while in the condensed state, which is different from their function in the active state and includes some effect on quantitative characters such as stature and intelligence and a role in sex determination. An alternative hypothesis is that a small part of the human X remains in the active state.

If any region of the X and Y carried homologous genes this region might well not be inactivated, as there would be no need for dosage compensation (Lyon 1962). In line with this Hayman & Martin (1965) found in two marsupial species with an XX/XY_1Y_2 sex chromosome mechanism, thought to have arisen by translocation between the X and an autosome, that the presumed autosomal part of the X was not late-replicating and was therefore presumably active. In the human X there is no really clearly synchronously replicating or non-condensed region. However, the cytological criteria are relatively very gross and it would be possible for a small active region to be present without detection. Moreover, insufficient is yet known of the causal relationships among the various properties of the inactive X or of inactive chromatin generally for it to be safe to assume that synchronous replication or lack of condensation necessarily implies activity. In cultures of human fibroblasts, for instance, about 30 % of cells lack a sex chromatin body. Therkelsen & Peterson (1967) measured the G-6-PD activity of such cultures, and compared it with the sex chromatin count at successive times. If the cells with no sex chromatin body had had all X chromosomes active the curves for these two properties should have tended towards the inverse of each other. In fact they did not and hence lack of visible condensation could not be taken to imply that all X chromosomes were active. Ferguson-Smith (1965), by studying the defects in individuals with one X partly deleted, rather than absent, showed that the abnormalities in human XO types were due to lack of the short arm of the X rather than the whole of it, and that corresponding genes might be present on the long arm of the Y. However, this does not indicate at what stage the genes act, nor whether they act in the condensed or active state, so that although the data are consistent with the presence in the human X and Y of a short homologous non-inactivated region, they do not exclude other explanations.

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Sex chromosome activity in germ cells

Yet another possibility is that incorrect dosage of the X chromosomes in the germ cells is the cause at least of the sterility. So far we have considered the activity of the X in somatic cells. Ohno (1967) and his colleagues noticed that there was no heteropyknotic X in oocytes of female mammals and it was therefore suggested that both X chromosomes might be active at this stage. Epstein (1969) measured two enzymes, G-6-PD, due to an X-linked gene, and lactate dehydrogenase (LDH), due to an autosomal gene, in oocytes of XO and XX mice. He found that the two types had equal activities of LDH, but that the G-6-PD activity in XX was double that in XO oocytes. He concluded that both X chromosomes were active in XX oocytes. The human XO ovary has oocytes at the foetal or newborn stage but lacks them later (Singh & Carr 1966; Conen & Glass 1963; and Polani, quoted by Hamerton 1968). Hence it is possible that a human oocyte requires the activity of two X chromosomes for survival.

The situation in male germ cells is less clear. Males of the XXY type, not only in man but also in mouse, cat, pig and ram, are sterile, with absence of germ cells from the testis (Cattanach 1961*b*; Thuline & Norby 1961; Breeuwsma 1968; Bruere, Marshall & Ward 1969). Is this again due to wrong X dosage? In later spermatogonial and further stages the X and Y both become condensed and do not form RNA when other chromosomes are doing so (Monesi 1965), i.e. they appear to be inactive. It is not yet entirely clear at what stage they become so. In the embryo the Y must presumably be active, since it determines the testis, but in somatic cells it is late replicating (Tiepolo, Fraccaro, Hultén, Lindsten, Mannini & Ming 1967) and presumably inactive.

Composite X chromosomes

In some mammals, there is a further complication. Only one arm or part of the X behaves as so far described and is singly active in somatic cells. The other arm is doubly late replicating and presumably inactive in somatic cells, of both the male and the female (Ohno 1967; Schmid 1967). The origin and functions of these doubly-condensed regions remains obscure.

Ohno's (1967) suggestion that X chromosomes of the composite type could be regarded as duplicate, triplicate or quadruplicate X chromosomes, with the doubly inactive regions consisting of replicates of the singly active regions, has become less likely, both with the increasing diversity of X chromosome types being discovered (see, for example, Schmid & Leppert 1968), and with Schmid's (1967) finding that the replication patterns of the doubly inactive regions are different from those of the singly active regions. Schmid considers that facultative heterochromatin, such as the singly active X region, has different origins and functions from those of constitutive heterochromatin, of which the doubly inactive regions form all or part in different species. The location of the constitutive heterochromatin on the X or on the autosomes can vary widely in closely related species, and some examples of species with simple and composite X chromosomes are shown in table 3. As well as this, the total amount of constitutive heterochromatin can vary, and Schmid therefore suggests that it has no developmental function but instead the evolutionary one of favouring the survival of chromosome exchanges. His suggestion of a lack of developmental function of this part of the X, at least in somatic cells, is favoured by the finding of Hill & Yunis (1967) that the doubly late replicating region of the golden hamster X had developed this property as early as the 8 cell stage of embryogenesis. However, this region is not heteropyknotic in oocytes (Ohno 1967) and not late-replicating in spermatogonia

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(Utakoji & Hsu 1965) and hence it may have some function in germ cells. If so, its wide variation from species to species would still remain to be explained. The finding of XO or XXY individuals in species with composite type X chromosomes might help to elucidate this point, which remains a problem for the future.

TABLE 3. EXAMPLES OF SIMPLE AND COMPOSITE X CHROMOSOMES IN MAMMALS

	simple X		composite X	
Rodentia				
mouse			golden hamster	
rat			chinese hamster	
guinea-pig	Schmid 1967		chinchilla	
			Microtus agrestis	Schmid 1967
			Microtus oregon	
			Microtus pennsylvanicus	Schmid 1967
			Rattus natalensis	Huang 1967
Artiodactyla				
cow			reindeer	Fraccaro *et al.* 1968
goat	Evans 1965		blackbuck }	Wurster, Benirschke
pig	Evans 1965		sitatunga }	& Noelke 1968
Other orders				
armadillo	Grinberg, Sullivan & Benirschke 1966			
cat				
dog				
human				
hedgehog	Citoler & Gropp 1969			
rabbit	Kinsey 1967			

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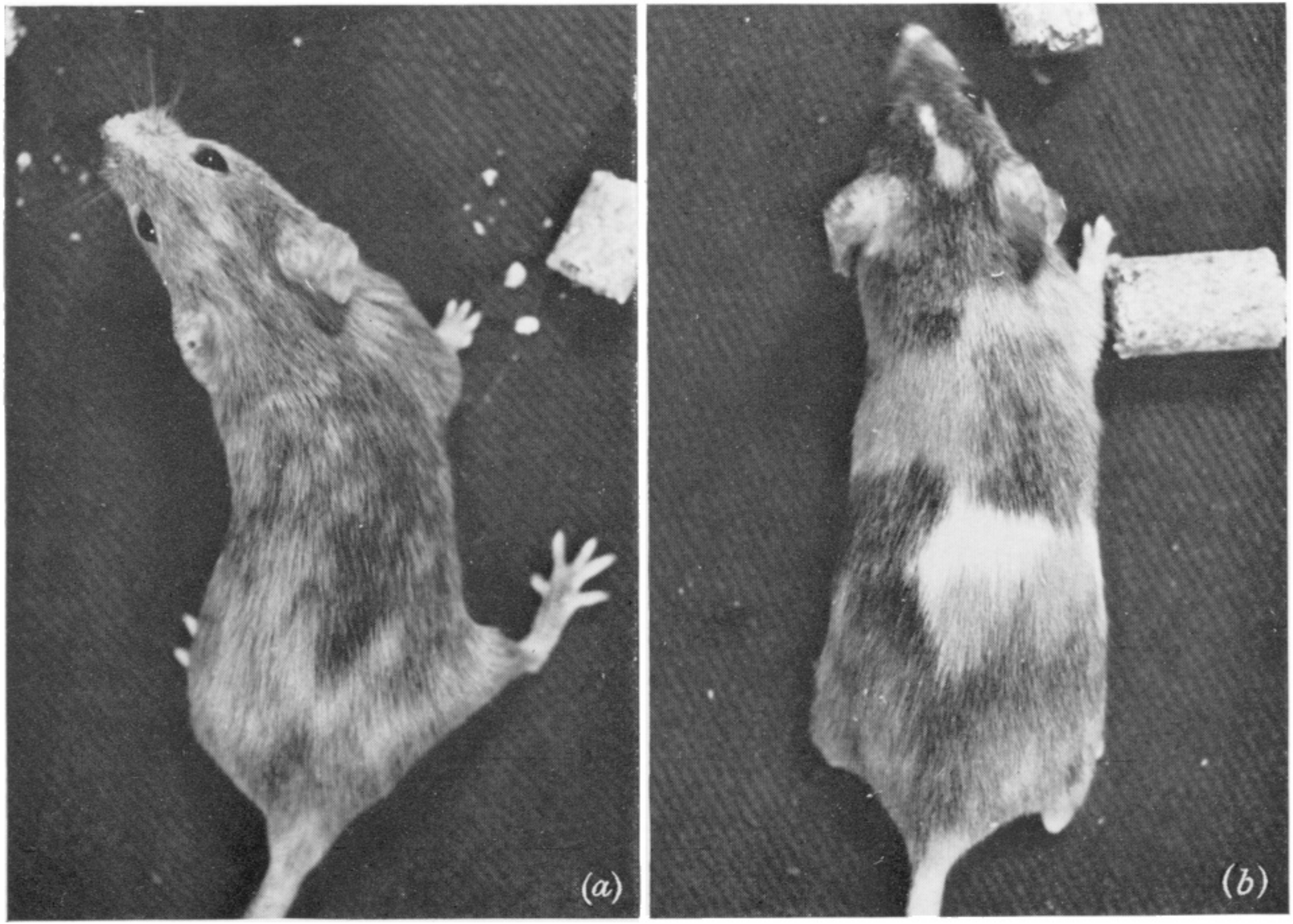

IGURE 2. Female mice heterozygous for Cattanach's translocation and variegated for pink-eye (*p*). (*b*) is also homozygous for piebald spotting (*s*). Note larger size of variegated patches in (*b*).

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